# FERAL HORSE AND BURRO FERTILITY CONTROL IN NEVADA: CONTRACEPTIVE VACCINE PILOT PROJECT

A Proposal Submitted to the
United States Department of the Interior
Bureau of Land Management
Nevada State Office

by

Kenneth W. Hunter, Jr., Sc.D.
Professor of Biology
Associate Vice President for Research
and Dean of the Graduate School
University of Nevada, Reno
Reno, Nevada

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The University of Nevada, Reno (UNR) is pleased to submit this proposal for a pilot project to evaluate a novel, single-injection contraceptive vaccine for fertility control in feral horses. Wild horses and burros in Nevada represent a magnificent natural resourse, but a resource that requires better management. We feel that an immunologic approach to fertility control represents a humane and cost effective way to manage wild horse and burro populations on public lands in Nevada and elsewhere.

While a variety of potential population management approaches have been discussed in the scientific community, the use of a zona pellucida-based vaccine in mares is perhaps the approach with the greatest present potential. In this proposed project, UNR will subcontract with the Medical College of Ohio for the services of Dr. John W. Turner and his colleagues Drs. Jay G. Kirkpatrick and Irwin K.M. Liu, acknowledged experts in the preparation and use of zona pellucida-based vaccines for fertility control. UNR will serve an administrative role, and provide oversight on the project through the following faculty committee:

Kenneth W. Hunter, Jr., Sc. D., Professor of Biology, Associate Vice President for Research and Dean of the Graduate School (Committee Chair)

Donald R. Hanks, D.V.M., Professor and Chair, School of Veterinary Medicine

Richard C. Simmonds, D.V.M., M.S., Director, Laboratory Animal Medicine

William G. Kvasnicka, D.V.M., Associate Professor of Veterinary Medicine and Extension Veterinarian

Ronald S. Pardini, Ph. D., Professor of Biochemistry and Associate Director, Nevada Agricultural Experiment Station

Duane L. Garner, Ph. D., Professor of Animal Science

This committee will meet periodically with the research team from the Medical College of Ohio and the Nevada Bureau of Land Management to plan and discuss the progress of the pilot fertility project.

The following section of this proposal outlines the experimental approach for the pilot fertility control project.

## INTRODUCTION

Feral horse management on western public lands is currently confined to the removal of excess horses. While we are not convinced that there is an actual overpopulation of horses in many areas, we recognize the need for improved, more effective management of feral horse populations. The removal of horses as the sole management effort, while seemingly effective at the time of removal, does not prevent the subsequent growth of the remaining population and insures that removal must continue year after year. Indeed, there is evidence that the removal of horses actually increases fecundity among those animals remaining behind and accelerates the growth of the population (Kirkpatrick and Turner 1991). In other words, removal alone addresses only the symptom of overpopulation (too many horses) and not the cause (reproduction).

An alternative approach is to limit reproduction, through some form of fertility control (see reviews by Kirkpatrick and Turner 1985, 1991; Turner and Kirkpatrick 1991). Toward that goal we have tested a contraceptive vaccine on feral horses which can limit the number of foals born to free-roaming mares. The major characteristics of this vaccine include (1) great effectiveness (> 95% effective), (2) remote delivery, which permits humane non-capture administration of the vaccine, (3) relative low cost, (4) no effects upon individual or social behavior of the target animals, (5) no effects upon pregnancies already in progress at the time of delivery, (6) reversible contraceptive action, and (7) no passage of the vaccine through the food chain or into the environment. These characteristics have been previously identified as required for successful feral horse contraception (Turner and Kirkpatrick 1986).

The vaccine, known as porcine (pig) zonae pellucidae, or PZP, satisfies these criteria. The zona pellucida is a non-cellular protein membrane which surrounds all mammalian eggs. In order for fertilization to occur, sperm must first bind to this membrane before they can penetrate the egg. The intramuscular injection of PZP into mares causes them to produce antibodies against the pig protein, but these antibodies also bind to the sperm attachment sites on the mares' eggs, thereby preventing sperm attachment and fertilization (for a review of the PZP vaccine see Paterson and Aitken 1990). Because only fertilization has been blocked, there are no hormonal manipulations which cause behavioral changes. Indeed, immunized mares remain together in their social groups, ovulate regularly during the breeding season, and permit mating behavior by the herd stallion, and in general reflect the social behavior of untreated feral horses (Kirkpatrick et al. 1990a).

This vaccine was originally tested on captive feral horses and prevented pregnancies in 13 of 14 treated mares (Liu et al. 1989). Following this, the vaccine was tested on free-roaming feral horses managed by the National Park Service (Kirkpatrick et al. 1990a).

The hallmarks of this first field test were successful remote delivery by means of barbless darts fired from a capture gun, a demonstration of the vaccine's effectiveness (no pregnancies among 26 treated mares vs. a 50% pregnancy rate among control mares), reversibility, and a demonstration of its safety for use in animals already pregnant at the time of inoculation. After four years of treatment over 60 "mare years" (i.e., the number of mares treated annually x the number of years treated) only a single foal has been born. This approach to fertility control in feral horses has been so effective that the National Park Service is already in the process of designing a management program built around this vaccine (personal communication, John Karish, Regional Scientist, Mid-Atlantic region, National Park Service). The effectiveness and safety of this contraceptive vaccine has been well documented and our own research group has tested the vaccine on a variety of other hoofstock, including white-tailed deer (Turner et al. 1992), sika, samabar, axis and muntjac deer and Himalayan tahr (Bronx Zoo), and West Caucasian tur (Toronto Zoo). Other investigators have demonstrated the effectiveness of the vaccine in a wide variety of non-human primates (Paterson and Aitken 1990) and even humans (Sacco 1987). Currently the vaccine is a candidate for development as a human contraceptive (Millar et al. 1989).

The vaccine has one major disadvantage at the present time. During the first year of administration of the vaccine, the mare must be inoculated twice, about three weeks apart. Contraceptive protection for subsequent years requires only a single booster inoculation (Kirkpatrick et al. 1992). Thus, the focus of current research efforts is to develop a one-inoculation vaccine which will permit one to two full years of contraception after a single administration. Basically, this will involve incorporating multiple doses of the PZP vaccine in a single inoculation in such a way that there is an initial release of some of the vaccine after injection and then a small but constant release of the remaining vaccine, similar to the way Contac® cold capsules work. A pilot study has already been carried out which has demonstrated the effectiveness of a continual release of the vaccine. This study, with domestic mares, employed a single injection followed by placement of an implant under the skin, which released the vaccine gradually over four weeks. Antibodies were produced in quantities which cause contraception and indicate that a one-inoculation sustained release system can be effective as a fertility inhibitor (see Figure 1).

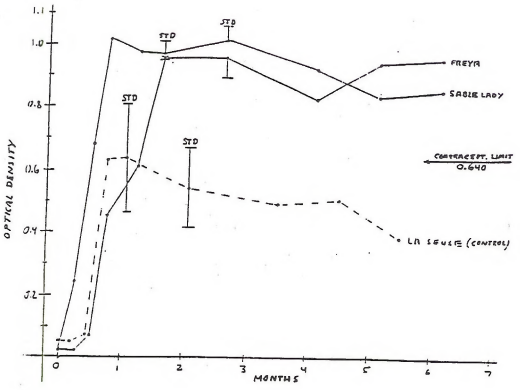


Fig. 1. Effect of sustained-release PZP vaccination in mares (Freya and Sable Lady) on anti-PZP antibody production. Vaccination consisted of bolus injection of 65 µg PZP and Freunds Complete Adjuvant (0.5 cc) followed by sustained PZP release (2.3 µg/day) over 28 days from a subcutaneously implanted osmotic minipump (Alzet, Inc.). Control (LaSense) received bolus PZP and Freund's Complete Adjuvant, but no pump. Plasma antibody titers are measured in optical density units. The lower limit of contraceptive efficiency determined from previous studies is 0.64 O.D.

With these encouraging data we have embarked upon the chemical engineering necessary to give us this same type of release pattern in a single injection. This is a collaborative effort between the Medical College of Ohio, Deaconess Research Institute, the University of California at Davis, and The Humane Society of the U.S.. The prototype timed-release preparation is already underway and we expect to have an initial testing of it in domestic mares completed by Fall of 1992. Additional funds are needed to complete this study, and this is the first of three studies for which we are requesting funding from your organization.

The second study for which we are requesting funding support is the development of a two-year contraceptive capability with a single injection. This will essentially involve an extension of the technology for the annual single-injection vaccine described above. It is obviously more time- and cost-efficient to deliver vaccine every other year instead of annually. The timed-release technology which is currently available must be evaluated for its specific application to the PZP vaccine. This approach involves formulating a single injection which contains the two-dose release sequence for the first year and a single dose released 9-12 months later for contraception during the second year. Long-term timed-release such as this, employing a process called microencapsulation, has been used for other applications (Eldridge et al. 1989). The high potency of the vaccine in small amounts makes it a very good candidate for permitting microencapsulation and still allowing remote delivery.

While the two studies described above will be primarily chemical engineering (with testing of antibody levels in domestic mares), the true test of the vaccine will require a field study. To accomplish this, the vaccine will be tested on free-roaming feral horses in Nevada. This third study, for which we are requesting funding, will be carried out in one or two herd areas mutually agreed upon by our research group and the agency or agencies appointed to make such decisions in Nevada. The field trials will evaluate effectiveness of the vaccine by pregnancy testing and foal counts. While remote delivery of vaccine in the field by darting from helicopter or at water holes is certainly a reasonable eventual goal, the proposed field trial will focus on injection in the chute following gathering. This will permit guarantee of scientific validity in terms of assured injection of vaccine and individual animal identification. Other field trial considerations such as cost, time, humaneness and safety will be monitored. While it is possible that the chemical engineering of the single-injection vaccine will be completed by Fall of 1992, we cannot guarantee this. Therefore, we propose two possible vaccination protocols for the 1992 gathering. If the single-shot vaccine is complete at that time, one half of the mares will be given a single injection and released while the remainder will be injected, retained for 3 weeks, reinjected and released. If the single-shot vaccine is not complete, then our current 2-injection procedure will be used on all mares. The proposed protocol will require maintaining horses in captivity for 3 weeks (without handling), but will permit successful vaccination and maintained flow of the project in the event that the single-injection engineering is delayed in completion. Because the second study (i.e., two-year capability) will probably not be complete by the time the initial field applications are needed the proposed first round of field testing will utilize only the prototype annual single-injection vaccine or current two-injection procedure.

While this proposal is brief and to the point, it is important in outlining crucial steps to enable large scale contraceptive vaccination of feral horses. We feel it is necessary to point out that the alternative available contraceptive technology - steroid

hormone implants - does not represent current technology nor does it satisfy basic criteria for humane treatment of animals. It is not cost-effective, safety for use in pregnant animals is still a question, behavioral effects are unknown, and steroid use is not likely to be permitted by the EPA because of possible environmental and food-chain contamination.

## RESEARCH PLAN

### Rationale

The purpose of this proposed research is three-fold and includes (1) development of a functional one-inoculation, one-year PZP contraceptive vaccine which can be delivered remotely for the regulation of free-roaming feral horses, (2) extension of that engineering technology to produce a one-inoculation PZP vaccine which will provide two-years of contraceptive protection, and (3) field test of the vaccine on free-roaming feral horses inhabiting public lands in Nevada.

### Objectives

The specific objectives of this proposed research include the following:

I. Development of the one-inoculation, one-year vaccine (in the form of MICROSPHERES).

   1. to determine if the PZP protein, or antigen, retains immunological activity during preparation for incorporation into microspheres,

   2. to engineer a sustained-release formulation for a one-inoculation PZP vaccine that will impart a full year of contraceptive protection, i.e., microspheres,

   3. to test the effectiveness of this one-inoculation, one-year vaccine to produce antibodies in domestic horses.

II. Development of a one-inoculation PZP vaccine which imparts two years of contraceptive protection (in the form of MICROCAPSULES).

   1. to determine whether the PZP antigen retains immunological activity during preparation for incorporation into microcapsules,

   2. to engineer a timed-release, pulsed-release formulation for a one-inoculation vaccine which will impart two-years of contraception,

   3. to test the effectiveness of the one inoculation, multiple year PZP vaccine to produce antibodies in domestic horses.

III. Remote field testing of the PZP vaccine in its current 2-injection form or as a single-injection prototype on free-roaming horses in Nevada. Note that additional field trials will be needed to complete PZP vaccine testing, and these will be addressed in a subsequent proposal.

Considerations in the development of a one-inoculation PZP vaccine

At the present time a minimum of two inoculations of the PZP vaccine, given three weeks apart, are necessary for effective contraception in horses. Despite the > 95% contraceptive effectiveness of the vaccine, the need for two inoculations greatly limits the usefulness of this approach for use in free-roaming horses. Thus, the first goal of this proposed research is to develop a method for delivering a single inoculation of PZP vaccine which will result in an immediate release of some of the vaccine antigen, and then a second release of the vaccine, either continuously for a month or so or as a pulsed release about 3 weeks later. Ideally, the one inoculation would also contain a third dose of the vaccine which would be released about one year later, thus resulting in contraceptive protection for two or more years.

There are two existing technologies which can immediately be applied to the PZP vaccine to meet these goals. The first is to bind the PZP antigen within an inert non-toxic polymer which, upon injection, will release the antigen continuously but slowly over some period of time. The chemical particles which contain the antigen are referred to as microspheres. The second technology is microencapsulation of the PZP antigen. This involves coating the antigen with a non-toxic material which, after injection, erodes away and also releases the antigen. Microcapsules differ from microspheres in that they cause a sharp, timed, pulsed release of the antigen rather than a sustained release (Maulding 1987).

The first timed-release approach involves the continuous, controlled release of PZP antigen imbedded within a microsphere matrix of poly (L-lactide) or copolymers of lactide and glycolide. This approach has been used for the delivery of a large number of drugs, including intramuscular and subdermal contraceptive agents, cancer chemotherapeutics and vaccines (Cowsar et al. 1985; Linhardt 1989; Staas et al. 1991). This methodology initially appeared less promising than microencapsulation (see below) because the process causes a continuous release of the antigen rather than pulses, and continuous release might result in tolerance to the antigen rather than production of high concentrations (titers) of antibodies. However, our preliminary study of continuous release of PZP antigen in mares (see page 4, Figure 1) has demonstrated that high titers of antibody, well above the contraceptive threshold, can be obtained by continuous release. These results make this approach very attractive. Microsphere release of a common protein (bovine serum albumin, or BSA) indicates that this process can duplicate the release we achieved with the implant (see Figure 2). The two real critical questions are whether or not the PZP protein will withstand the chemical process required for incorporation into

microspheres and whether microsphered PZP vaccine will work in vivo.

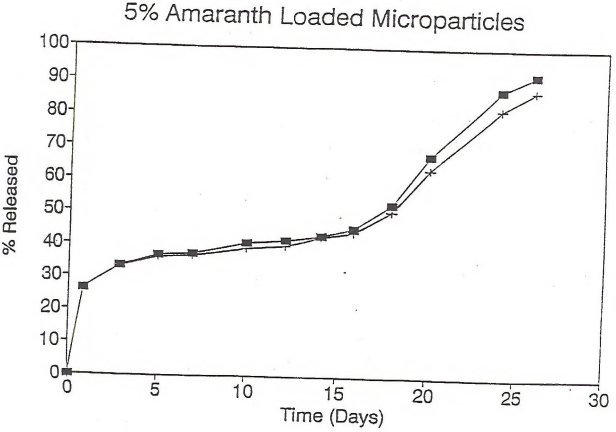


Figure 2. Release rates for bovine serum albumin from lactide pellets.

The technology to produce a one-inoculation PZP vaccine by microencapsulation also already exists. Several protein vaccines have been microencapsulated for oral delivery in humans (Eldridge et al. 1989), and there is a high probability that the same thing can be done for the intramuscular injection of PZP antigen. In the microencapsulation process the protein antigen, PZP in this case, is coated with a non-toxic polymer material, producing small capsules about the size of talcum powder grains. Upon injection into the animal the coating begins to erode. When erosion is complete, the PZP is released. We have previously used this very technique - microencapsulation - to deliver contraceptive steroid hormones to feral horses (see Kirkpatrick et al. 1982; Turner and Kirkpatrick 1982; Turner and Kirkpatrick, 1991). Long-term release rates for vaccines incorporated into microcapsules have been reported to be maintained for up to 2 years (Staas et al., 1991) and we expect that the same sort of sustained release can be achieved with the PZP antigen. Once again, the two critical questions are whether the antigen can withstand the chemical process required for incorporation into microcapsules and whether

the preparation works in vivo.

There are several laboratories which can microencapsulate protein molecules. The most established microencapsulation laboratories in the U.S. are Southern Research Institute (Birmingham, AL), and Medisorb Technologies (Cincinnati, OH). Their approach is to coat the protein antigen with a non-toxic biodegradable coating (D,L-lactide and D,L-lactide co-glycolide) which, on contact with tissue fluids breaks down into harmless products such as carbon dioxide and lactic acid (Redding et al. 1988). When the coating erodes, the protein antigen is released and stimulates the animal to produce antibodies which will bind to its own zonae pellucidae, on its own eggs, and thereby block fertilization.

**Considerations for field tests of the one-inoculation vaccine:**

Regardless of the success of the chemical engineering necessary to develop the one-inoculation vaccine, the ultimate measure of success in this project will be the effectiveness of inhibiting fertility in PZP-treated free-roaming feral horses in Nevada. Thus, the second major component of this project is to test the one-inoculation vaccine under field conditions. This will involve selection of an appropriate herd area in Nevada, gathering of horses at the appropriate time administration of PZP vaccine or placebo to identified mares in the field and monitoring of these mares for pregnancy and foaling.

## METHODS

**STUDY 1**

**PZP Microsphere Development:** This work will be performed under subcontract, in the laboratory of R. Linhardt, at the University of Iowa. Approximately 3.0 mg of PZP will be obtained from I.M.K. Liu, at the University of California, Davis. The PZP will be tested for its ability to withstand concentrating, lyophilization, organic solvent exposure, desalting, and heat exposure. These tests are necessary to determine if the PZP antigen can withstand the actual chemical processes necessary for incorporation into microspheres. Retention of the PZP's ability to raise antibodies will be determined by a procedure known as western blot electrophoresis, using PZP anti-horse antibodies already prepared at U.C.-Davis, by M. Bernoco. If the PZP retains its ability to raise antibodies, the next step is to actually incorporate 65 µg doses of PZP, along with an appropriate adjuvant, into microspheres. These microspheres will then be injected into 3 domestic horses, at the Equine Reproduction Laboratory at U.C.-Davis. Periodic blood samples will be collected to determine if the horses are raising antibodies against the microspheres.

**Microsphere preparation and in vivo testing:** If antibody titers sufficient for contraception are obtained, the most promising formulation will be prepared for injection into a larger number of domestic horses. Preparation will be by R. Linhardt and associates using procedures previously described (Wang et al., 1990, 1991).

PZP release rates will be designed on the basis of previously effective doses in horses, such that 65 μg is released initially and 65-90 μg is released continuously thereafter over one month. Also, Freund's Complete Adjuvant (FCA) will be used based on previous success with this adjuvant in horses. Adjuvants are compounds which, when given with a vaccine, cause the target animals' immune systems to produce very high concentrations of antibodies against the vaccine. A study is already underway which is investigating the possible use of other adjuvants which have minimal side effects and maximum antibody responses. This adjuvant study, conducted by us and funded in part by the American Association of Zoological Parks and Aquariums (AAZPA) will run parallel to our research on a one-inoculation PZP vaccine and will provide valuable information for identifying sound adjuvants for use with the PZP vaccine in horses. The expanded horse study will utilize domestic horses at the Equine Reproduction Laboratory at U.C.-Davis, and will be supervised by Dr. I.K.M. Liu.

**Study Design**

Group 1 - Free PZP bolus and PZP microspheres + FCA (n=5)

Group 2 - PZP microspheres + FCA (n=5)

Group 3 - Empty (or BSA-loaded) microspheres + FCA (n=5)

**Study Schedule**

1. Immunization injection 6 weeks prior to onset of breeding is preferred.

2. Blood sample prior to inoculation and monthly post-inoculation for antibody titer measurement.

3. Fecal and/or urine samples prior to inoculation and monthly post-inoculation to determine pregnancy. This will be performed by J. F. Kirkpatrick, Deaconess Research Institute, Billings, MT and will provide information regarding contraceptive efficacy eight months prior to expected foaling time, thereby permitting maximum lead time for designing the next phase of the research.

4. All mares will be placed with fertile stallions and the above schedule of collections and tests will be carried out until antibody titers drop below the contraceptive threshold (previously determined by I.K.M. Liu et al. (1989); all animals will be monitored for general health and physical condition during the study.

Part of Study 1 is already underway as a collaborative effort between The Humane Society of the U.S., the Medical College of Ohio, Deaconess Research Institute, the University of California at Davis, and the University of Iowa.

STUDY 2

Two-year contraceptive vaccine with a single inoculation (microencapsulation): This is primarily a chemical engineering study and will involve subcontracting with one of several companies (Southern Research Institute, Birmingham, AL; Medisorb Technologies, Inc., Cincinnati, OH) to formulate the PZP preparation according to the timed-release schedule we request. Testing of antibody-stimulation characteristics will be performed by I.K.M. Liu. Basically this research will follow the same steps described above for the one-year microsphere inoculation, i.e., (1) testing of the antigen for its ability to withstand the process of microencapsulation, (2) incorporation of PZP antigen into microcapsules designed to give a release one-month, and 10 months after injection, and (3) in vivo testing of microcapsules in domestic horses. Depending upon the start-up date, this projected research will permit in vivo testing in domestic mares by Fall of 1992.

STUDY 3

Field study of one-inoculation PZP vaccine

Selection of field site: A feral horse herd in Nevada will be identified and agreed upon for field test of the PZP vaccine. Selection will require mutual agreement by our research group, the Bureau of Land Management and the State of Nevada. Selection criteria will include (1) topography suitable for testing, (2) herd size suitable for testing, (3) available background data regarding fertility rates, mortality rates, and population dynamics which will permit reasonable population modelling, and (4) available logistical support (housing, transportation, etc.). The site presently under most serious consideration is the combined herd management areas of Antelope and Antelope Valley in eastern Nevada. All agencies with regulatory authority over the test animals must agree, in writing, that only horse gathers or removals associated with the experimental design of this study will be conducted during the course of these studies.

For the selected feral horse population several population parameters must be established before treatment can begin. First, the desired population effect must be determined. This can be stated as a question; do we wish to achieve negative growth, zero growth, or some predetermined low growth rate? Second, once the desired population effect has been decided upon, we must determine what percentage of sexually mature mares must be treated in order to achieve the population effect, i.e., 60%, 70%, etc. Finally, we suspect that there are differential fecundity rates among mares with foals (yearlings at the time of treatment) and those without foals. Recent evidence from feral horses in California (J. W. Turner, unpublished data) and on a barrier island (Kirkpatrick and Turner 1991) indicate that mares without foals are more likely to be pregnant than those with foals and are less likely to become pregnant the next year. In the herd or herds to be treated in the proposed studies contraceptive treatment efforts will include as

many mares with foals as possible. The determination of the population goals, size of the target treatment population, and which individual animals provide the best opportunity for contraceptive success are the domain of population modelling (we suggest Dr. Walt Conley, New Mexico State University, for this input), and these parameters will be assessed before actual treatment begins. As a first estimate regarding the Antelope (n=468) and Antelope Valley (n=540) HMA's, based on discussion with informed BLM personnel, an "n" of 100-140 mars in the 5-9 year age group may be available for the study. Prior to beginning the field test it must be demonstrated that the herd is in reasonably good nutritional state, 2) the range is in fair to good forage condition with reliable water availability and that adequate gathering/holding capabilities exist to carry out the study.

**Treatment Procedures:** Gathering by bands is preferred to insure family integrity. However, our experience has been that gathered horses which have been separated from their bands and then released back into their home range area have good probability of relocating and rejoining their original band. Gathered females will be individually identified by freeze-brand marking. Pregnancy can be determined via urine sample testing on site (Roser and Lofstedt 1989) and injection of selected mares can be accomplished by jab-stick in chutes, or blowpipes in the corrals.

PZP antigen for these field tests will be produced by I.K.M. Liu, at U.C.-Davis. The PZP-loaded microspheres and/or microcapsules will be formulated and produced by the appropriate subcontractor (Linhardt, University of Iowa; Southern Research Institute; Medisorb Technologies, Inc.). Delivery of PZP vaccine to horses will be conducted/monitored by members of our research group.

Only healthy mares (as determined by our research team veterinarian) will be used in the study. Treatment of mares will be done in a blind study initiated in fall/winter based on the successful protocol developed in the course of the Assateague Island studies. Pending availability of single-injection vaccine and 140 mares for treatment, the following groups and numbers will be included: 2-injection PZP (55), 2-injection placebo (15), 1-injection PZP 956), 1-injection placebo (15). The 2-injection groups are essential in this study as a reference base with which to compare the 1-injection preparation. As stated in the Introduction section, Introduction section, if the 1-injection prep is not available by the time the treatments must be done, all mares will be given the 2-injection protocol. This will insure a viable field trial of PZP vaccine in 1992. Observations will be made of the horses during the ensuing breeding season in order to document that social structure is intact and to determine if there is any significant change in behavior. Essentially we are interested in whether or not harem groups are intact, whether mares are being attended by the stallions, and whether mares are displaying clinical signs of behavioral estrus. Additionally, a certain number of treated mares with unique identifying markings will be photographed for later identification. This will be important for

determining the duration of contraceptive effects.

Although the initial test will utilize gathered horses and direct injection of vaccine, an important consideration for vaccine delivery in the future is remote darting. Therefore, preliminary evaluation of this issue will be undertaken in the proposed studies. Capture gun technology is designed primarily for immobilizing animals, and not for remote delivery of drugs. Modifications of equipment and techniques of delivery are required to deliver drugs remotely to free-roaming animals and our experience with feral horses on Assateague Island has provided a great deal of experience in this area. There are currently several brands and models of capture guns and self-injecting darts which can be considered candidates for this work. These include the Pax-Arms rifle, Pneu-dart, Inc., and the Teleinject system. Additionally, Dr. Lee Simmons, of the Omaha Zoo, can provide custom capture rifles. Each of these instruments has advantages and disadvantages and it is our intention, in the course of this study, to evaluate all systems and seek appropriate modifications in order to achieve the greatest success. It is important to remember that, even when the one-inoculation vaccine is available, it will do little good if we can't get it into the horses.

Pregnancy diagnosis: At the time of the gather (1992) blood/fecal samples will be collected for pregnancy testing. Mares given 2-injections of PZP will also be blood sampled at the time of 2nd injection for antibody titer testing. Between August and November (1993) following the breeding season urine and/or fecal samples will be collected from a statistically valid sample of the treated and untreated populations. The urine and fecal samples will be collected as described by Kirkpatrick et al. (1988, 1991a), and measured for pregnancy-dependent estrone conjugates and non-specific progesterone metabolites as described by Kirkpatrick et al. (1988, 1990b, 1991b). The establishment of pregnancy rates is important because foaling rates do not always provide accurate pictures of contraceptive effectiveness. Fetal loss and early foal mortality (the latter witnessed by J. W. Turner among California feral horses where foals are subject to lion predation) can confound the measurement of contraceptive effectiveness; early pregnancy determination can provide a more accurate picture. And, while pregnancy detection is important, in keeping with our research group's concern for the safety and humane treatment of horses, remote pregnancy testing is an integral part of a complete hands-off approach to fertility control.

Experimental controls: Previous work with feral horses on Assateague Island national Seashore has documented the lack of contraceptive effects of placebo vaccination upon control animals. However, the validity of the proposed field test will be insured by including placebo controls for each type of treatment. The control preps will consist of an emulsion of phosphate buffer solution and Freund's adjuvant.

Treatment Evaluation: Field studies of contraception can be evaluated and measured for success or failure in different ways.

Our approach is to document the pharmacological success of contraception. This will be accomplished by comparing pregnancy and foaling rates among treated and untreated mares. This is a major focus of the present proposal and will be carried out by our research group. While it will ultimately be necessary to understand what the effects of contraception may be upon the population dynamics, this is beyond the scope of our proposed studies. Nonetheless, the proposed field trial can provide the beginning of a data base for population models to determine to what degree immunocontraception may alter the demographic dynamics and size of a feral horse herd.

**Animal care**: All research conducted in the course of this project will be subject to review by the appropriate animal research committees of the three institutions involved (Medical College of Ohio, Deaconess Research Institute, and the University of California at Davis), and will be conducted only after approval by these committees. The regulations surrounding animal care standards for wild or free-roaming species are not clear. However, our group will apply the standards for domestic animals to the treatment of all horses in this study, whether domestic or free-roaming.

**Education and public relations**: Our research group's experience with the highly visible and successful Assateague Island feral horse contraception study has made it extremely clear that a serious attempt must be made to keep the public informed and to provide open and honest dialogue with the media. The Assateague horses are the most visible - and perhaps most adored - feral horses in North America, and embarking upon the immunocontraceptive research project carried with it a certain amount of risk. In order to keep the public informed at each step of the project, the National Park Service conducted an extensive educational program. This involved the print media, local and national network TV, and on-site programs. After six years of research with this highly visible herd, which has some 700,000 visitors come to view it each year, there has been absolutely no public resistance and overwhelming public support, including animal protection groups. The key elements of this successful relationship with the public were careful documentation of each step of the research and willingness and efforts to share this information with the public. It is our intention to do the same thing with this proposed research. An experienced public relations expert will be retained by the research team on a consulting basis, to design an appropriate public relations program and to develop the necessary materials for disseminating information. Our research group has never killed or even seriously injured a horse in the course of 18 years of research; we are as proud of that as we are of our contraceptive success. We feel that the public must be able to view our work and the care we take if this approach to the control of feral animal populations is to become accepted. No information will be released without going through the consultant resource, who must have approval of the research team scientists for any information release.

## INVESTIGATOR EXPERIENCE

The three investigators are Dr. John W. Turner, Jr., Department of Physiology and Biophysics, Medical College of Ohio, Toledo, Dr. Jay F. Kirkpatrick, Deaconess Research Institute, Billings, MT, and Dr. Irwin K. M. Liu, University of California, Davis, and the collaborating agency is the Humane Society of the U.S. Drs. Turner and Kirkpatrick have been involved in studies of the biology of feral horses for 18 years. These studies have focused on hormonal contraception and immunocontraception of both stallions and mares and culminated in the successful immunocontraception of the Assateague horses. Funding for these projects have come from a variety of source but primarily from the Department of the Interior, through the Bureau of Land Management (Contract YA-512-CT) and the National Park Service (Contract CA-1600-30005). In addition to contraceptive studies these two investigators have also pioneered non-capture methodologies for detecting pregnancy and monitoring ovarian function among free-roaming feral horses in order to develop a complete "hands-off" technology for the control of feral horse reproduction. Both investigators will personally devote a significant portion of their time to this project. Specifically, Dr. Turner will oversee the chemical engineering of the one-inoculation vaccine and play a significant role in designing and conducting the field testing of the vaccine. Dr. Kirkpatrick will be in charge of remote pregnancy detection, evaluation of vaccine delivery equipment, development of the public relations program and will participate in field tests. Together these investigators are responsible for 28 published scientific articles relating to feral horse biology and contraception, as well as numerous articles in the popular press. Dr. I.M.K. Liu is an equine immunologist in the School of Veterinary Medicine at U.C.-Davis. Dr. Liu was responsible for originally determining that the PZP vaccine is effective in horses and he has extensive experience testing this vaccine with feral horses living on sanctuaries. He will be in charge of vaccine production and antibody testing. All investigators will be present for the gathering and treatment of horses. Academic credentials and qualifications for the three co-investigators are provided in the appendix.

## PROJECT EVALUATION

The project will be evaluated periodically at several check points, as well as at the conclusion. The check points, derived from the stated goals include (1) <u>in vivo</u> testing of the microsphere PZP vaccine (evaluation criteria = antibody concentrations and pregnancy rates), (2) <u>in vivo</u> testing of the microcapsule PZP vaccine (evaluation criteria = antibody concentrations and pregnancy rates), (3) effectiveness in the field of the vaccine delivered to feral horses percent of treated vs. control mares which produce foals. All endpoint evaluations are measurable and will result in data which can be tested for significance.

## LITERATURE CITED


Cowsar, D.R., T.R. Tice, R.M. Gilly, & J.P. English 1985. Poly(lactide-co-glycolide) microcapsules for controlled release of steroids. in: Widder, K.J., & R. Green (Eds.), Methods in: Enzymology: Drug and Enzyme Targeting, Academic Press, Orlando, FL, pp 101-116.

Eldridge, J.H., R.M. Gilly, J.K. Stass, Z. Moldozeanu, J.K. Meulbroek, & T.R. Tice 1989. Biodegradable capsules: vaccine delivery systems for oral immunization. Curr. Topics Microbiol. Immunol. 146:59-66.

Kirkpatrick, J.F., J.W. Turner, Jr., & A. Perkins 1982. Reversible fertility control in feral horses. J. Equine Vet. Sci. 2:114-118.

Kirkpatrick, J.F., & J.W. Turner, Jr. 1985. Chemical fertility control and wildlife management. BioScience 35:485-491.

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Kirkpatrick, J.F., I.M.K. Liu, & J.W. Turner, Jr. 1990a. Remotely-delivered immunocontraception in feral horses. Wildl. Soc. Bull. 18:326-330.

Kirkpatrick, J.F., B.L. Lasley, & S.E. Shideler 1990b. Urinary steroid evaluations to monitor ovarian function in exotic ungulates. VII. Urinary progesterone metabolites in the Equidae assessed by immunoassay. Zoo Biol. 9:341-348.

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Kirkpatrick, J.F., S.E. Shideler, B.L. Lasley, & J.W. Turner, Jr. 1991b. Pregnancy determination in uncaptured feral horses by means of fecal steroid conjugates. Theriogenology 35:753-759.

Kirkpatrick, J.F., & J.W. Turner, Jr. 1991. Compensatory reproduction among feral horses. J. Wildl. Manage. 55(4):649-652.

Kirkpatrick, J.F., & J.W. Turner, Jr. 1991. Reversible fertility control in non-domestic animals. J. Zoo Wildl. Med. 22(4):392-408.

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Linhardt, R.J. 1989. Biodegradable polymers for the controlled release of drugs. in: Controlled Release of Drugs, Polymer and Aggregate Systems, Rosnoff, M. (ed.), VCH Publishers, New York, pp 53-95.

Liu, I.M.K., M. Bernoco, & M. Feldman 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. J. Reprod. Fert. 85:19-29.

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Stass, J.K., J.H. Eldridge, J.D. Morgan, O.B. Finch, T.R. Tice, & R.M. Gilley 1991. Microsphere vaccines: Enhanced immune response through adjuvant effect and multiple-pulse release capability. Proc. Intern. Symp. Control Rel. Bioact. Mater. 18.

Tolson, N.D., K.M. Charlton, G.A. Casey, M.K. Knowles, C.E. Rupprecht, K.F. Lawson, & J.B. Campbell 1988. Immunization of foxes against rabies with vaccinia recombinant virus expressing rabies glycoprotein. Virology 102:297-301.

Turner, J.W., Jr., & J.F. Kirkpatrick 1982. Steroids, behaviour and fertility control in feral stallions in the field. J. Reprod. Fert. (Suppl. 32):79-87.

Turner, J.W., Jr., & J.F. Kirkpatrick 1986. Fertility control as a management tool for feral horse populations. J. Equine Vet. Sci. 6:278-284.

Turner, J.W., Jr., & J.F. Kirkpatrick 1991. New developments in feral horse contraception and their potential application to wildlife. Wildl. Soc. Bull. 19:350-359.

Turner, J.W., Jr., I.M.K. Liu, & J.F. Kirkpatrick 1992. Remotely-delivered immunocontraception of captive white-tailed deer. J. Wildl. Manage. 56(1):154-157.

Wang, H.T., H. Palmer, R.J. Linhardt, D.R. Glanagan, & E. Schmidt 1990. Degradation of poly(ester) microspheres. Biomaterial 11:679-685.

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## PROPOSED PROJECT BUDGET

**SECTION I. UNIVERSITY OF NEVADA, RENO (UNR) BUDGET**

**A. Personnel**

Principal Investigator (K. Hunter) $5,831
(P.I. commitment to project is 5% of total time, plus 19% fringe benefits)

**B. Travel**

Travel from university to study site via university vehicle for P.I. and members of oversight committee $500

| | |
|---|---|
| DIRECT COST TOTAL | $6,331 |
| INDIRECT COST TOTAL* | $11,075 |
| TOTAL UNR COSTS | $17,406 |

* includes indirect costs on first $25,000 of subcontract to Medical College of Ohio

**SECTION II. PROPOSED SUBCONTRACT BUDGET (MEDICAL COLLEGE OF OHIO)**

**PART I. Chemical Engineering (Microsphere/Microencapsulation) Study**

**A. Personnel**

Principal Investigator (J. Turner) $14,523.00
(P.I. commitment to this project is 20% of full-time effort. Plus 34% fringe benefits)

Co-Principal Investigator (J. Kirkpatrick) $ 6,100.00
(Co-P.I. commitment to this project is 10% of full-time effort. Plus 22% fringe benefits)

Research Associate $18,000.00
(Salary for preparation of PZP. 30% of full-time effort. Plus 34% fringe benefits)

Laboratory/Secretarial Assistance $14,472.00
(Part-time, $9/hr. X 24 hrs/wk (Medical College of Ohio) x 40 wks, plus 34% fringe benefits)

Laboratory Technician $16,080.00
(Part-time, $10/hr. X 30 hrs/wk X 40 wks, plus 34% fringe benefits)

SUBTOTAL $69,175.00

B. Microsphere and Microcapsule Formulation and Testing

| | |
|---|---|
| Viability testing of vaccine for the formulations | $ 5,000.00 |
| Timed-release vaccine preparation | $16,000.00 |
| Vaccine release characteristics testing | $14,000.00 |
| In vivo testing of the timed-release vaccine | $15,000.00 |
| SUBTOTAL | $50,000.00 |

C. Equipment

| | |
|---|---|
| Dionex Pulsed Electrochemical Detector and electrode for HPLC analysis of urine/feces | $ 7,900.00 |
| Reciprocal shaker for urine/fecal extractions | $ 2,000.00 |
| SUBTOTAL | $ 9,900.00 |

D. Supplies

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|---|---|
| Supplies for PZP preparation, antibody monitoring, blood collection, horse maintenance | $ 5,800.00 |

E. Communications

| | |
|---|---|
| Phone, fax, mailing, copying | $ 1,600.00 |

F. Consultants

| | |
|---|---|
| Public Relations Costs | $ 6,000.00 |

G. Travel

| | |
|---|---|
| Principal Investigator (J. Turner): | |
| Toledo to site for microsphere preparation | $ 900.00 |
| Toledo to site for microencapsulation preparation (2 trips) | $ 1,800.00 |
| Toledo to site for timed-release vaccine testing in vivo | $ 900.00 |
| SUBTOTAL | $ 17,000.00 |

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|---|---|
| DIRECT SUBCONTRACT COST SUM (PART I) | $146,075.00 |
| MCO Indirect Costs (20% of above) | $ 29,215.00 |
| **TOTAL SUBCONTRACT COSTS (PART I)** | **$175,290.00** |

**Part II. Field Trials Study**

The costs of field trials will depend on the range site selected. Since conditions and tactical support elements vary

considerable from range to range, it is not possible to make a reliable cost projection. However, there are some aspects of the field trial costs which are fixed and an overall cost estimate can be made, assuming up to 140 mares will be treated.

The following budget is divided into 2 parts. Section A shows costs which will be provided to the Medical College of Ohio, and Section B shows costs which will be covered within the operating budget of the BLM.

**Section A. (Costs Provided to MCO)**

1. Personnel costs for 2 field technicians (students) to carry out the field monitoring of the PZP-treated and placebo horses, including urine/fecal sample collections for pregnancy testing and behavioral monitoring. Vehicles, fuel, and housing to be provided by BLM.

| | |
|---|---|
| $8.00/hr X 8 hr/day X 100 days X 2 persons (plus 34% fringe benefits) | $ 17,152.00 |
| $15.00/person per diem X 100 days | $ 3,000.00 |
| SUBTOTAL | $ 20,152.00 |

2. Equipment

| | |
|---|---|
| a. Horse identification by videotape has proven superior in our studies, and we recommend that each monitoring person have such capability. Cost for freeze-frame videocamera is about $1,500. Sony TR-101 handycam $1,500 X 2 | $ 3,000.00 |
| b. Binoculars $200 X 2 | $ 400.00 |
| c. Spotting scope $300 X 2 | $ 600.00 |
| SUBTOTAL | $ 4,000.00 |

3. Supplies and communications, i.e., for sample collection and storage, horse monitoring, phone and mailing — $ 2,500.00

4. The cost of vaccine will depend on the results of the Microsphere-Microencapsulation study and on the number of horses to be treated. A conservative estimate is $35/horse. If the experimental phase is successful a larger scale PZP preparation system will greatly reduce the cost per horse.
Based on 110 mares treated plus 35 reserve doses.

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|---|---|
| Estimated Subtotal | $ 5,075.00 |

5. Cost of pregnancy testing will be approximately $15.00 per sample including shipping and assay and will be based on 140 mares (30 control and 110 experimental)

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|---|---|
| Estimated Subtotal | $ 2,100.00 |
| SUBTOTAL | $ 9,675.00 |

6. Travel

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|---|---|
| Travel by Turner, Kirkpatrick, Liu and assistant to range site to perform vaccinations. | $ 3,000.00 |
| Travel by Dr. Turner or Kirkpatrick to verify foal counts and evaluate horse population in study range. | $ 2,000.00 |
| Travel by 2 field technicians to range site. | $ 2,000.00 |
| SUBTOTAL | $ 7,000.00 |

| | |
|---|---|
| Total Direct Costs for Section A. | $40,827.00 |
| MCO Indirect Costs (20%) | $ 8,165.00 |
| **Total Costs for Section A.** | **$48,992.00** |

**Section B. (Costs Covered Directly by BLM)**

1. All helicopter costs: for initial observations of range, gathers of horses for PZP treatment and post-treatment monitoring (including flyovers for horses identifications and foal counts).

2. All equipment, supplies and personnel costs for gathering of horses and maintenance of captive horses, including corrals, freeze-branding, disease testing, veterinary care, feed, water/feed transport.

3. Provision of 4 X 4 vehicles and fuel for all research activities during the field trial.

**PROJECT BUDGET SUMMARY**

| | |
|---|---|
| **University of Nevada, Reno Costs** | **$17,406** |
| **Subcontract Costs (Medical College of Ohio)** | **$224,282** |
| **Total Project Costs** | **$241,688** |

# CURRICULUM VITAE

**Kenneth W. Hunter, Jr.**

3460 Southampton Drive
Reno, Nevada 89509
(702) 324-1815

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*Present Positions*:

Associate Vice President for Research
and Dean of the Graduate School
University of Nevada, Reno
239 Getchell Library
Reno, Nevada 89557-0035
Tel: (702) 784-6869
FAX: (702) 784-6064
e-mail: *khunter@unssun.nevada.edu*

Professor of Microbiology
University of Nevada School of Medicine
Department of Microbiology

Professor of Biology
University of Nevada, Reno
College of Arts & Sciences
Department of Biology

*Recent Past Positions*:

| | |
|---|---|
| 1986-1989 | President and Chief Executive Officer<br>Biotronic Systems Corporation<br>Rockville, Maryland 20850<br>(Presently, Chairman of the Board) |
| 1985-1989 | Chief Scientist<br>Westinghouse Bio-Analytic Systems Co.<br>Madison, Pennsylvania |
| 1982-1989 | Founder and Executive Vice President<br>ANTECH Consultants, Inc.<br>Rockville, Maryland |

*Education*:

| | |
|---|---|
| 1977 | The Johns Hopkins University<br>School of Hygiene and Public Health<br>Department of Pathobiology<br>Sc. D., Immunology-Parasitology |
| 1972/73 | Arizona State University<br>Department of Zoology<br>B.A., Biology<br>M.S., Zoology |

*Academic Experience*:

| | |
|---|---|
| 1986-89 | Adjunct Associate Professor<br>Uniformed Services University<br>of the Health Sciences<br>F. Edward Hebert School of Medicine<br>Departments of Pediatrics and<br>Preventive Medicine/Biometrics<br>Bethesda, Maryland |
| 1982-86 | Associate Professor of Pediatrics<br>and Preventive Medicine/Biometrics<br>Director of Pediatric Research<br>Uniformed Services University<br>of the Health Sciences<br>F. Edward Hebert School of Medicine<br>Department of Pediatrics |
| 1979-82 | Research Assistant Professor<br>(Primary Appointment)<br>Uniformed Services University<br>of the Health Sciences<br>F. Edward Hebert School of Medicine<br>Department of Pediatrics<br>(Infectious Disease Section) |
| 1978-79 | Post-Doctoral Fellowship<br>Uniformed Services University<br>of the Health Sciences<br>F. Edward Hebert School of Medicine |

Department of Medicine
(Infectious Disease Division)

*Graduate Grants and Fellowships::*

| | |
|---|---|
| 1976 | Visiting Research Associate<br>Agency for International Development<br>Malaria Research Project<br>University of New Mexico<br>Albuquerque, New Mexico |
| 1975 | Immunology Research Grant<br>The John W. Graham Fund<br>The Johns Hopkins University |
| 1974-1977 | Pre-Doctoral Fellowship<br>Parasitology-Medical Entomology<br>National Institutes of Health<br>National Institute of Allergy and Infectious Diseases |
| 1973 | Research Collaboratorship<br>United States Department of Agriculture<br>Agriculture Research Service<br>Western Cotton Research Laboratory<br>Division of Entomology<br>Phoenix, Arizona |

*Professional Societies::*

American Association for the Advancement of Science
American Association of Immunologists
Helminthological Society of Washington
Tropical Medicine Association of Washington
American Society of Tropical Medicine and Hygiene
American Society of Clinical Pathology
Association of Official Analytical Chemists

*University Committees*:

Uniformed Services University of the Health Sciences (USUHS) Biohazard Suite Coordinating Committee, Chairman
Armed Forces Radiobiology Research Institute (AFRRI)/USUHS Radionuclide and X-Ray Safety Committee
USUHS Research Proposal Merit Review Committee
USUHS Faculty Senate Research Policy Committee
University of Nevada, Reno (UNR) Biomedical Human Subjects Committee
UNR Social/Behavioral Human Subjects Committee
UNR Biohazards, Controlled Substances, and Dangerous Materials Committee, (Recombinant DNA Subcommittee)
UNR Intellectual Property Committee
UNR Institutional Animal Care and Use Committee
UNR Radiation Safety Advisory Board
UNR Graduate Council
University and Community College System of Nevada Research Affairs Committee

*UNR Representative To*:

Western Association of Graduate Schools
National Association of State Universities and Land Grant Colleges, Council on Research Policy and Graduate Education
Intermountain University Research Administrators
Council of Graduate Schools
Council on Government Relations
Society of Research Administrators

*Advisory Groups*:

Technical Consultant, NSTA/NASA
Space Shuttle Student Involvement Project
Goddard Space Flight Center
Greenbelt, MD, 1984

Scientific Advisory Panel
European Journal of Epidemiology, 1984-

Advisory Panel
U.S. Environmental Protection Agency
Biomarkers Peer Review and Panel, 1986

Governor's Task Force on
Regional Economic Development
Greater Washington Region, 1988

The Johns Hopkins University Society of Alumni
Career Opportunities Committee, 1988

Advisory Panel
Congress of the United States
Office of Technology Assessment
"Technologies to Detect Pesticide
Residues in Food", 1988

Member, Board of Directors
Montgomery County High Technology
Council, Inc. 1988-1991

College of American Pathologists
Future Technology Committee, 1988-1989

American Society of Clinical Pathologists
New Technology Committee, 1988-1989

Elected Advocate
Maryland Conference on Small Business
1988-1990

State of Maryland, Governor's Office
Partnership for Workforce Quality
Advisory Board, 1989

The Johns Hopkins University
Montgomery County Center
Advisory Board, 1989

Nevada Innovation, Technology,
and Entreprenurial Council
Member, Board of Trustees, 1989-
Vice President, 1990-

Western Industrial Nevada
Member, 1990-

Nevada Industry, Science, Engineering
and Technology Organization, Inc.
Member, Board of Trustees, 1990-

Nevada State Development Corporation
Board of Trustees, 1991-

Nevada Space Grant
Associate Director, 1991-

Nevada State EPSCoR Committee
Member, 1990-

*Research Interests*:

Gene Regulation in Leishmania
Immunoregulatory Functions in Malaria
Medical and Agricultural Entomology
Monoclonal Antibodies for Chemical Haptens
Somatic Cell Genetics and Human Hybridomas
Biosensors and Molecular Electronics

*Past Research Support*:

K.W. Hunter, Principal Investigator
*Temperature-Induced Transformation in Leishmania*
USUHS RDT&E Grant
February 1980 - February 1982
$102,732

K.W. Hunter, Principal Investigator
*Detection of Organophosphates Using Immunologic Methods*
U.S. Army (AARADCOM) Chemical Systems Laboratory
May 1981 - September 1985
$599,000

K.W. Hunter, Principal Investigator
*Human Monoclonal Antibodies to Malarial Antigens*
U.S. Agency for International Development (USAID)
July 1981 - January 1983
$35,000

K.W. Hunter, Principal Investigator
*Somatic Cell Cloning of Mouse and Human*
*Somanase and Butyrylcholinesterase*

U.S. Army Chemical Research and Development Center
March 1983 - March 1985
$200,000

K.W. Hunter, Principal Investigator
*Evaluation of the Prophylactic Potential of Monoclonal Anti-Soman Antibodies*
U.S. Army Medical Research & Development Command
May 1983 - September 1986
$526,000

K.W. Hunter, Principal Investigator
*Preparation and Characterization of Mouse and Human Monoclonal Antibodies to Botulinum Toxins*
U.S. Army Medical Research & Development Command
April 1982 - September 1984
$136,000

K.W. Hunter, Principal Investigator
*Rapid Biosensor Assay for AIDS Virus Antibodies*
National Institute of Allergy and Infectious Disease
Small Business Innovation Research Program
February 1989-June 1990 : Phase I $50,000
June 1990- : Phase II $500,000

*Awards*:

USUHS Outstanding Performance Awards, 1979-1985
Department of the Army, Special Commendation, 1984
USUHS Distinguished Service Medal, 1987
Who's Who in Science and Technology, 1991

*Courses Taught*:

Diagnostic Parasitology and Medical Zoology
Medical Microbiology (Immunology and Parasitology)
Medical Entomology
Epidemiology
Preventive Medicine and Public Health

## PUBLICATIONS


1. **Hunter, K.W.** 1973. Effect of the parasite *Copidosoma truncatellum* (Dalman) on food consumption of *Trichoplusia ni* (Hubner) larvae. Masters Thesis, Arizona State University, Tempe, Arizona.

2. **Hunter, K.W.** and A. Stoner. 1975. *Copdiosoma truncatellum*: Effect of parasitization on food consumption of *Trichoplusia ni*. Environ. Entomol. 4:381-382.

3. **Hunter, K.W.** and A.C. Bartlett. 1975. Chromosome number of the parasitic encyrtid *Copidosoma truncatellum* (Dalman). Ann. Entomol. Soc. Amer. 68:61-61.

4. **Hunter, K.W.** 1977. Serum opsonic activity in rodent malaria. Doctoral Thesis, The Johns Hopkins University, Baltimore, MD.

5. **Hunter, K.W.** 1979. Searching behavior of *Hippodamia convergens* larvae (Coccinellidae: Coleoptera). Psyche 85:249-254.

6. **Hunter, K.W.**, F.D. Finkelman, G.T. Strickland, P.C. Sayles,and I. Scher. 1979. Defective resistance to *Plasmodium yoelii* in CBA/N mice. J. Immunol. 123:133-137.

7. **Hunter, K.W.**, A.R. Campbell, and P.C. Sayles. 1979. Human infestation by cat fleas, *Ctenocephalides felis* (Siphonaptera: Pulicidae), from suburban raccoons. J. Med. Entomol. 16:547.

8. **Hunter, K.W.**, J.A. Winkelstein, and T.W. Simpson. 1979. Serum opsonic activity in rodent malaria: functional and immunochemical characteristics in vitro. J. Immunol. 123:2582-2587.

9. **Hunter, K.W.**, G.W. Fischer, P.C. Sayles, and G.T. Strickland. 1979. Increased resistance to malarial infection following treatment with the immunostimulator levamisole. Curr. Chemother. Infect. Dis. 2:1099-1101.

10. Mease, A.D., G.W. Fischer, **K.W. Hunter**, and F.B. Ruymann. 1980. Decreased phytohemagglutinin-induced aggregation and C5a-induced chemotaxis of newborn neutrophils. Pediat. Res. 14:142-146.

11. **Hunter, K.W.**, F.D. Finkelman, G.T. Strickland, P.C. Sayles, and I. Scher. 1980. Murine malaria: Analysis of erythrocyte surface-bound immunoglobulin by flow microfluorimetry. J. Immunol. 125:169-174.

12. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and A.D. Mease. 1980. Diminished bacterial defenses with intralipid. Lancet 2:8I9-820.

13. Strickland, G.T., and **K.W. Hunter**. 1980. The use of immunopotentiators in malaria. Int. J. Nuc. Med. Biol. 7:133-140.

14. Fischer, G.W., **K.W. Hunter**, and S.R. Wilson. 1980. Intralipid and reticuloendothelial clearance. Lancet 1:1300.

15. Strickland, G.T., and **K.W. Hunter**. Red cell antibodies in malaria: Immunity or Autoimmunity? *In* The Host-Invader Interplay, H. Van den Bossche ed., Elsevier/North-Holland Biomedical Press, Amsterdam, 1980.

16. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and S.A. Henson. The role of antibody in Group B streptococcal disease. *In* Immunoglobulins: Characteristics and Used of Intravenous Preparations. B.M. Alving, J.S. Finlayson, eds. Washington, D.C. U.S. Government Printing Office, 1980:DHEW publication No. (FDA)-80-9005.

17. **Hunter, K.W.**, T.M. Folks, P.C. Sayles, and G.T. Strickland. 1981. Early enhancement followed by suppression of natural killer cell activity during murine malarial infections. Immunol. Lett., 2:209-212.

18. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and V.G. Hemming. 1981. Modified immune serum globulin. Lancet 1:271.

19. **Hunter, K.W.**, G.W. Fischer, P.C. Sayles, and G.T. Strickland.1981. Levamisole: Potentiation of primary immunoglobulin M antibody responses in suckling rats. Immunopharmacology 3:117-127.

20. Sayles, P.C., **K.W. Hunter**, E.E. Stafford, and L.D. Hendricks. 1981. Antibody response to *Leishmania mexicana* in the African white-tailed rat (*Mystromys albicaudatus*). J. Parasitol. 67:585-586.

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## PATENTS

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2. U.S. Patent No. 4,865,972 entitled, "Antibody-Based Bioassay for Enzyme Inducing Chemicals", September 12, 1989.

3. U.S. Patent No. 5,057,598 entitled, "Monoclonal Antibodies Reactive With Endotoxin Core", October 1991, with Matthew Pollack, M.D. (including continuation and foreign filings).

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## ABSTRACTS

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## SPECIAL INVITED PRESENTATIONS

United States Army Biomedical Laboratory Symposium, The Immunology of Organophosphorus Compounds, Aberdeen Proving Ground, MD, May 20, 1981. Presentation entitled, *"The Use of Monoclonal Antibodies and Enzyme Immunoassay for Detection of Organophosphorus Compounds".*

First Annual U.S. Army Armament Research and Development Command Technical Conference, Picatinny Arsenal, Dover, NJ, June 16-18, 1981. Presentation entitled, *"Detection of Paraoxon".*

1981 Chemical Systems Laboratory Scientific Conference on Chemical Defense Research. Aberdeen Proving Ground, MD, 16-20 November 1981. Presentation entitled, *"Immunologic Method for the Rapid Detection of Soman (GD)".*

Research Colloquium, Chemical Systems Laboratory, Aberdeen Proving Ground, MD., July 9, 1982. Presentation entitled, *"Immunodetection of Soman".*

1982 Chemical Systems Laboratory Scientific Conference on Chemical Defense Research, Aberdeen Proving Ground, MD, 15-18 November 1982. Presentation entitled, *"Inhibition of the Anticholinesterase Activity of Soman by Monoclonal Antibody".*

1983 International Symposium on Protection Against Chemical Warfare Agents, Stockholm, Sweden, June 6-9, 1983. Presentation (A.A. Brimfield) entitled, *"Fine Specificity of Anti-Soman Monoclonal Antibodies".*

Research Colloquim. Chemical Research and Development Center, Aberdeen Proving Ground, MD, October 7, 1983. Presentation entitled, *"Immunodetection of the Trichothecene Mycotoxin T-2".*

European Symposium on "New Horizons in Microbiology", April 26-29, 1984. Presentation entitled,*"Protection Against Haemophilus influenzae type B by Antibodies to the Capsular Polysaccharide".*

1984 FASEB Summer Research Conference on Diagnosis, Toxicity, and Therapy of Trichothecene Mycotoxicosis, June 25-29, Vermont Academy, Saxtons River, VT. Presentation entitled, *"Immunodetection of T-2 Toxin Using Monoclonal Antibody".*

1984 Chemical Research and Development Center Scientific Conference on Chemical Defense Research. Aberdeen Proving Ground, MD, 12-16 November 1984. Presentation (R.F. Schuman) entitled, *"Secretion of Acetylcholinesterase by a Hybrid Cell Line".*

USUHS Continuing Medical Education, Japan and Okinawa Tour, May 1984. *"Presentations on Advances in Medical Biotechnology and Tropical Medicine".*

U.S. Environmental Protection Agency, Peer Review and Biomarkers Panel Meeting, San Jose, CA. June 24-26, 1986. Presentation entitled, *"Detection of Chemical Warfare Agents Using Monoclonal Antibody-Based Immunoassays".*

Research Colloquium: New Methods in the Detection of Environmentally Important Compounds. U.S. Environmental Protection Agency, Research Triangle Park, NC. November 20-21, 1986. Presentation entitled, *"Monoclonal Antibody-Based Immunoassay for Toxic Chemicals".*

IEEE Conference on Synthetic Microstructures in Biological Research, Airlie, VA. March 23-26, 1986. Presentation (A.L. Newman) entitled, *"Development of an Antibody-Modulated Planar Capacitive Sensor".*

College of American Pathologists, 1987. Annual Meeting, Molokai, Hawaii. January 15-18, 1987. Presentation entitled, *"Technological Advances in Bedside Monitoring: Biosensors".*

National Food Processors Association, Atlantic City, NJ, March 12, 1987. Presentation entitled, *"Monoclonal Antibody-Based Immunoassays for Toxic Organic Compounds".*

American Chemical Society, Division of Agrochemicals Special Conference III, Biotechnology in Crop Protection, June 28-July 3, 1987, Snowbird, Utah, Presentation (W.L. Stanbro) entitled, *"Interfacing of Hybridoma and Microelectronics for Use in Environmental Analysis".*

American Pathology Foundation, 10th Annual Meeting, July 11-14, 1987, San Diego, CA. Presentation entitled, *"Biosensors and Telemetry for Near Instantaneous Viral Diagnosis: Hepatitis and Acquired Immune Deficiency Syndrome".*

Society for Industrial Microbiology, Annual Meeting (August 9-15, 1987, Baltimore, MD. Presentation entitled, *"Capacitive Affinity Sensor: A New Multi-Purpose Biosensor".*

EPRI PCB Seminar, October 6-9, 1987, Kansas City, MO. Presentation (R.F. Schuman) entitled, *"Ultrasensitive Bioassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)".*

American Pathology Foundation Seminar "Managing for Profit: Taking Advantage of New Techniques and Emerging Opportunities in Pathology", February 26-27, 1988, Charleston, SC. Presentation entitled, *"Biosensors and Telemetry in Bedside Testing".*

American Society of Clinical Pathology Symposium: Present and Future Applications of New Technology in Anatomical and Clinical Pathology, April 18, 1988, Kansas City, MO. Presentation entitled, *"Advances in Bedside Monitoring: Biosensors".*

Association of Official Analytical Chemists Biotechnology Symposium, August 29 September 1, 1988. Palm Beach, FL. Presentation entitled, *"Biosensors: A New Dimension for Immunoassays".*

Symposium: Developments in Biosensor Technology, July 25, 1988, New Orleans, LA. Presentation entitled, *"Capacitive Affinity Sensors: Multi-Purpose Biosensors".*

International Biosensors '88 Symposium, August 22-23, 1988, Washington, D.C. Presentation entitled, *"Commercializing Biosensors: The Transition from the Research Laboratory to the Marketplace".*

American Society of Clinical Pathologists/College of American Pathologists Spring Meeting, March 11-16, 1989, Chicago, IL. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classification and Evolution".*

International Biosensors '89 Symposium, October 16-17, 1989, New Orleans, LA., Presentation entitled, *"Identifying Market Niches for Biosensors".*

American Society of Clinical Pathologists/College of American Pathologists Fall Meeting, October 28-November 3, 1989, Washington, D.C. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classification and Evolution".*

American Society of Clinical Pathologists/College of American Pathologists Fall Meeting, October 20-24, 1990, Dallas, TX. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classifcation and Evolution".*